Seminar. Brandeis University. April 23, 1958

Alteration of Gene action by Genetic Mechanisms.

I. The Problem: What types of genetic mechanisms bring about abrupt and distinct changes in gene action in specific cells during development.

2. Why problem arises: Recently become aware that genetic changes occur to nuclei during development and are probably associated with developmental processes.

3. Development of gene theory: First half of century:
   a). Based mainly on selected mutants
   b). Mutants segregated in clear-cut fashion in heredity.
   c). Mutants -- inconsequential types:
      Drosophila - white eyes, yellow body color, curled wings, folked bristles, etc.
      Maize - Anthocyanin pigments in plant and kernel, starch types in kernel, etc.
   c). Arrangements of change in linear order in chromosome.
   d). Repeated occurrences of change at one locus -- same phenotype altered.
   e). Chromosome behavior -- reduplication - sister chromatids alike: Based on germe line for continuation of mutant type.
   f). Postulate: All nuclei of individual have same genetic constitution: Extrapolation. Not direct evidence.
   g). Effect on embryology: Confusion.
      Weiss - 194 : Clear statement of confusion: Realized some differentiation related to change in potential of nucleus.
         Tried to ask geneticists for a mechanism.
         Tried to find evidence in genetics for differentiation of nuclei.

4. What was known of differention of nuclei:
   a). Germ line - set aside early - protected from change
   b). Soma: Chromosome changes:
      Poly ploid nuclei
      Polytene nuclei
      Loss of certain chromosomes in certain nuclei
      Non-disjunctions of certain chromosomes at s ecific divisions
      Loss of whole set of chromosomes in certain nuclei
      Loss of part of a chromosome - specific in certain division
      Segregation of polytene chromosomes -- reduction somatic
      Result: No direct effect on geneticists -- did not alter views.

   c). Changes in gene action known:
      Position effect in Drosophila - change in position of gene with respect to heterochromatin and euchromatin -- variegation in individual -- differential action of genes in different cells.
      Variegation, so-called mutable genes: Known in all organisms. Wide-spread phenomena. Exceedingly difficult to analyse in many cases. Relagated to peculiar, unimportant phenomena. No effect on genetic theory.
      Pattern alleles - Found in many forms.
         R alleles in maize. Control where and how much anthocyanin developed in kernel and plant: Example:
            Color in plant, not in kernel
            Color in kernel, not in plant
            Color in plant -- at very restricted regions for each allele.

Lady Beetle: Pigment spots. Alleles. Each specific
In both cases - genes for pigment should be present in all cells
Why functioning in only certain ones to produce particular pattern?

5. Recent developments:
   a). Elegant proof of genetic changes during development without change in chromosome complement that is visible: Frogs Eggs. Briggs and King

   b). Rhynchosciara and Chironomous -- change at gene level but visible in chromosome -- will return to this.

II. Recent developments in understanding genic materials.

   a). Genetic materials - DNA molecuel - long.
   b). All information codeddin the structure of the DNA molecule
   c). Mutations - selected types -- arise from change at particular site in molecule: Change in enzyme. Relation of DNA to protein synthesis.

   d). The Problem: What is the chromosome - Very large body indeed.

   What is the nucleus -- its function.

III. The Nucleus: An organ of the cell. Functions to control the production and distribution of the gene products.

   a). At any one stage - linear organization of chromosome précise:
   Morphology clear-cut: [redacted] Pachytene in maize.
   What does this organization mean?

   b). Heterochromatin and euchromatin -- Knobs. What do they do?

   c). Evidence of action of genes in Rhynchosciara. Breuer and Pavan.

      (1) Eggs - laid at one time
      (2) Development - all go along together
      (3) Chromosome organization in different tissues: Polytene type - like Drosophila Salivaries
      (4). Comarisons of bands in different tissues

   d). Change in activity of different genes and different times in development

   e). Class I. Increase in activity of band for period and subsides. Return to previous appearance.

   Class II. Increase in DNA of band. Return to band much enlarged
   Permant change at gene locus. In soma, not germ line.

   f), Importance of observations for differential gene action in development-
   Turning on-and-off of gene action - no permanent change at locus.
   " " " " " " " - permanent alteration at locus.

g). In study of controlled changes in gene action in maize -- have examples of both types. Will discribe.

IV. Early History of study of change in gene action during development in maize

a). Began 1944 - Subjected plants to new situation during early development: One arm of one chromosome -- constantly altered during each cell division for period in early development.

b). Plants self pollinated. Probeny grown from about 400 plants.

c). In progeny -- very large number of cases of instability of gene expression during development -- variogation, "mutable loci", "mutable genes". Large number of different loci involved.

d). An extraordinary experience -- All types of plant and kernel characters affected by such changes -- Anthocyanin, chlorophyll, starch types, growth types, etc.

e). One event in common for all: The time when change in gene action would occur -- genetically controlled. The progeny of two sister cells could differ in type of control: Examples:

(1) One - changed rate of subsequent mutation - increased
Sister cell - decreased rate.

1, 2, 3 4

(2) One cell - mutation
Sister cell - changed rate of mutation **Slide** ~~2, 3, 4, 5, 6~~

f). One basic pattern -- controlled turning on and off of gene action during development. Genetic mechanism responsible for the changes in gene action.
Basic pattern for all cases, regardless of primary phenotype gene affected.

g). Why was basic pattern of gene behavior so drastically upset for so many genes as a consequence of an alteration affecting only one are of one chromosome? What condistions were distrubed by this that altered timing of gene action and for so many different genes?

V. Extensive study of one system of control of gene action - The DS- Ac system.

a). Two elements, each distinguishable because of phenotypes each produces-- like any so-called gene mutant-- control action of gene.

b). Unlike the stationary genes -- the DNA carrying elements-- these elements do not remain in one positions in the chromosome complement. Can transpose from one location to another without losing identity in process. Like genes but change their locations in the complement.

c). Inheritance can be followed as readily as any mutant. Linkages, positions in chromosomes, etc. Transposition frequencies and times and positions may be followed.

d). Both elements can cause a change in the chromosome - physical change- that may be observed. Like Phynchosciara - permant changes. Class II.

e). Transposition of Ds element to locus of known gene -- change in gene action results: C Ds to $C^{Ds} = c$

f). As long as Ac absent, c is a good recessive mutation. No evidence of mutation to C. As good as any known mutation in maize.

g). When Ac present -- mutations to C occur. Occur at certain times in development and in certain cells.

h). Control of time of mutation -- depends on Ac. As a whole the higher the dose of Ac, the later the time of occurrence of mutation. ~~Slides~~ Controls pattern - when gene acts and in what cells.

i). The mutation process - due to change in Ds element, not in genes at C locus. Ds sometimes removed when change occurs.

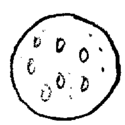
j). Because of transposition, could get cases of Ds entering different known gene loci. Many obtained:

C	Sh	Bz_1	Wx	A_1	A_2	Bz_2	Su
Chr.9				3	5	1	4

k). The Alleles of Ac and Ds. Different alleles (formerly called states) of both Ds and Ac can be recognized. Like alleles of known genes in that they are clear expressions but not related to one position in chromosome.

One allele of Ac: Pattern with all Ds mutants:

Photo



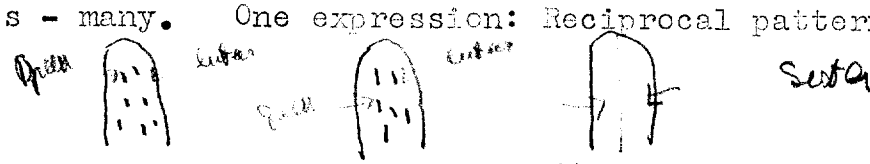
Exceedingly precise control. Very stable type of expression.

Another allele - always produced change of gene action in one particular cell. Precise control of time and cell in which change would occur.

VI. Investigation of another system of elements -- Spm

1. History of detection: One of the early cases - luteus, yellow plant. chlorophyll.

a). Mutation types - many. One expression: Reciprocal patterns: Sector



b). Inheritance behavior -- very confusing. Many changes in plant to different expressions and controls. No clear cut inheritance behavior that was readily analysible -- at that time.

c). In course of study -- appeared another mutable in culture having mutable lu -- A to a - mutable. Anthocyanin in plant and kernel.

d). Study of a -m. Much like lu. Very difficult to comprehend the changes in gene action and the mechanism responsible. High state of confusion.

c). In course of study of a_2-m, cross: $A_1\ A_1 \times a_1{}^s$ $\underline{\underline{A_2}}$

One kernel on ear - colorless with A_1 spots.
Plant grown from it. Origin of $a_1{}^{m1}$

2. Study of $a_1{}^m$ - simple.

a). Assume element entered A_1 (evidence of this later). Change from A to a-m

b) Mutations to A or alleles of A in presence of another element - Spm.

c), Original type of mutation pattern produced by a_1-m <u>Slide</u> ~~10~~ 5

d). Missing kernel - appearance. <u>Slide</u> ~~11~~ 6

Spm present - spots of A in colorless background - kernel
Streaks of A in green background - plant.

Spm absent - deep color in kernel and plant. Constant in behavior.

e). Spm - controls suppression of gene action and induces mutation.
Mutants in germ line -- stable in system.

f). Some other Alleles of a_2 isolated.: <u>Slide</u> ~~12~~ 7
<u>Slide</u> ~~13~~ 8
<u>Slide</u> ~~14~~ 9
<u>Slide</u> ~~15~~ 10

g). Inheritance of alleles: Combined:

<u>Slide</u> ~~16~~ 11
<u>Slide</u> ~~17~~ 12

h). Allele of Spm - Spm-w. Reduced capacity for inhibition of gene action and for mutation:

Plant colored - slowly. Kernel colorless, few A specks only.

Recessive to Spm-standard.

VII. Return to study of a_2-m, from which a_1-m arose. Same system?

a). Found same general system in some cultures of $a_1{}^{m-1}$ and very confused type of behavior in others.

b). Spm element present in the readily analysible cultures but did not know what was present in others.

b). Introduced Spm from a_m cultures into a_2-m of both types -- immediately cleared up behavior of $a_2{}^m$ Followed exactly that for $a_1{}^m$.

d). Proof of same Spm element controlling mutations in both -- long set of experiments.

e). What about behavior in a_2m culturs that were not analysible: Why?

f). Types of difficulties: Inheritance of variegation and expression

(1). Female - colored. x a_2^s -- all kernels colored except one or two -- ~~xxxx~~ colorless background, few to many specks of color.

Pollen - to a_2 - colorless background, many large spots of color.

(2). Main ear on plant - no variegation. Tillers segregated distinct ratios or peculiar ratios.

(3) Two distinct classes of alleles. Behavior different.

VIII. The solution of the a_2^m situation:

Two classes of alleles of a_2^m.

Class I. Spm present - gene action turned on and off but no change in gene. When Spm absent or turned off A_2 expression. Will return to this.

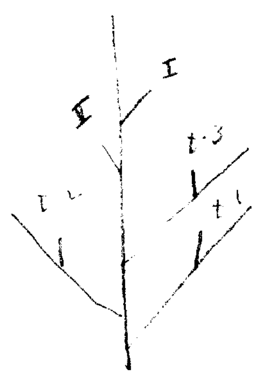
Class II. In absence of Spm - pale color in plant and kernel
In presence of Spm - mutations to A_2.

The Spm present -- its action cyclic - turned on and off in series.

The Standard Spm - turned on in plant throughout growth of plant and formation of gametes - turned off in some cells of endosperm.

The Spm in a_2^m - cycle shifts: Off and on. Time of turning off and on and time of mutation related.

Example: Plant 7109B-1 Allele - Class I Wx + / wx Spm



Example: Plant Allele Class II

Method of detecting presence of inactive Spm:

Active Spm ♀ Endosperm 2 Spm × 1 active ♂ = 3 active
Mod " " " 2 modifier × " " = 3 "

Wx + / wx Spm - active x a^2 wx 1 Active Spm

" + / wx Spm - inactive x " " " " "

Proved change in cycle action of Spm without change in position.
Proved presence of inactive Spm when not seen in plant or kernels.

IX. Meaning of study -- now have known system - control of turning on and off of gene action at specific time and in specific cells - produces very specail patterns of behavior of gene in plant and kernel.

Like Class I change in Rhynchosciara

Same system can operate to control permanent changes in gene action - like Class II of Rhynchosciara.

Can get various types of control of gene action by stabilizing cycle of Spm to any one type -- like Standard Spm stabilization.

Interchange of Spm types from a_2^m and a_1^m -- showed same behavior in control of gene action in both.

Combinations and permutations of alleles of two types of controlling elements - can get almost any time of control of gene action differentially in different tissues of same organisms.

System of control of gene action during development may not be too complex to appreciate.